10 Rec'd PCT/PTO 30 OCT 2001

FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 27656/37821

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): 10/018437

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP00/03708	26 April 2000	30 April 1999

TITLE OF INVENTION: **OLIGONUCLEOTIDE DERIVATIVES DIRECTED AGAINST HUMAN BCL-XL AND HUMAN BCL-2 MRNA**

APPLICANT(S) FOR DO/EO/US: **ZANGEMEISTER-WITTKE, UWE et al.**

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include itens (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

Return receipt post card; Sequence Listing in paper form; Sequence Statement

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U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/018437	PCT/EP00/03708	27656/37821

24. The following fees are submitted:.	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :		
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1040.00**		
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$890.00**		
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$740.00**		
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$710.00**		
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$890.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$0.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	26 - 20 =	6	x $18.00	**$108.00**	
Independent claims	1 - 3 =	0	x $84.00	**$0.00**	
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$998.00**	
☐ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				**$0.00**	
SUBTOTAL =				**$998.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				**$0.00**	
TOTAL NATIONAL FEE =				**$998.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				**$0.00**	
TOTAL FEES ENCLOSED =				**$998.00**	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of **$998.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **13-2855** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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Customer Number: 04743
MARSHALL, GERSTEIN & BORUN
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SIGNATURE

Jeffrey S. Sharp
NAME

31,879
REGISTRATION NUMBER

30 October 2001
DATE

10019437 .060302
531 Rec'd PCT/F... 30 OCT 2001

PATENT APPLICATION
Attorney Docket 27656/37821

IN THE UNITED STATES PATENT
AND TRADEMARK OFFICE

Applicants:	)	"EXPRESS MAIL" mailing label No. EL564464932US
Zangemeister-Wittke, *et al.*	)	
	)	Date of Deposit: October 30, 2001
Serial No.: Unknown	)	
	)	I hereby certify that this paper (or fee) is being deposited with the United States Postal Service "EXPRESS MAIL POST OFFICE TO ADDRESSEE" service under 37 C.F.R. §1.10 on the date indicated above and is addressed to the Commissioner for Patents, Washington, D.C. 20231
Filed: Herewith	)	
For: Oligonucleotide Derivatives Directed Against Human bcl-xL and Human bcl-2 mRNA	)	
Group Art Unit: Unknown	)	
Examiner: Unknown	)	Richard Zimmermann

PRELIMINARY AMENDMENT ACCOMPANYING FILING
OF NATIONAL STAGE APPLICATION UNDER 35 U.S.C. 371

Commissioner for Patents
Washington, D.C. 20231

Sir:

This is a preliminary amendment to the above-identified application. Please enter the following amendments.

In the Claims:

Please amend claims 3, 4, 6, 7, 8, 19, 20, 21, 22, 23, 24, and 25 as set out below:

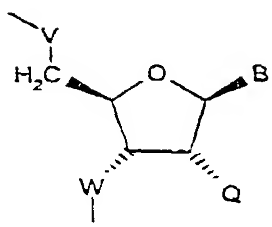
3. (Amended) The oligonucleotide derivative according to claim 1 comprising a base sequence which is complementary to at least a part of the said region of the human bcl-xL mRNA or the human bcl-2 mRNA, or wherein such base sequence contains up to 3 mispairing building blocks, or wherein such base sequence contains up to 3 abasic building blocks.

4. (Amended) The oligonucleotide derivative according to claim 1 having a length of 8 to 25 consecutive building blocks.

6. (Amended) The oligonucleotide derivative according to claim 3 wherein said base sequence is selected from the group consisting of the base sequence 5′ - AAGGCATCCCAGCCTCCGTT-3′ (SEQ. ID NO.: 3), the base sequence 5′-AAAGTATCCCAGCCGCCGTT-3′ (SEQ ID NO: 4), and the base sequence 5′-AAAGCATCCCAGCCTCCGTT-3′ (SEQ ID NO: 5).

7. (Amended) The oligonucleotide derivative according to claim 1, consisting of a base sequence selected from the group consisting of the base sequence 5′ - AAGGCATCCCAGCCTCCGTT-3′ (SEQ ID NO: 3), the base sequence 5′-AAAGTATCCCAGCCGCCGTT-3′ (SEQ ID NO: 4) and the base sequence 5′-AAAGCATCCCAGCCTCCGTT-3′ (SEQ ID NO: 5).

8. (Amended) The oligonucleotide derivative according to claim 1, comprising at least one building block of formula (I).



(I),

wherein

Q is H, $-OCH_3$, $-O(CH_2CH_2)_nOCH_3$, or $-OCH_2CH_2NR_1R_2$, wherein R_1 and R_2 are, independently of each other, H or $-CH_3$, and wherein n is 1, 2 or 3;

V and W are, independently of each other, the same or different radicals of an internucleosidic bridging group selected from the following group: 5'-O-P(O)(OH)-O-3' (phosphodiester), 5'-O-P(O)(SH)-O-3' (phosphorothioate), 5'-O-P(S)(SH)-O-3' (phosphodithioate), 5'-O-P(O)(CH_3)-O-3' (methylphosphonate), 5'-O-P(O)(NH-R_7)-O-3' (phosphoamidate) in which R_7 is C_1-C_3alkyl, 5'-O-P(O)(OR_8)-O-3' (phosphotriester) in which R_8 is C_1-C_3alkyl, 5'-O-S(O)$_2$-CH_2-3' (sulfonate), 5'-O-S(O)$_2$-NH-3' (sulfamate), 5'-NH-S(O)$_2$-CH_2-3' (sulfonamide), 5'-CH_2-S(O)$_2$-CH_2-3' (sulfone), 5'-O-S(O)-O-3' (sulfite), 5'-CH_2-S(O)-CH_2-3' (sulfoxide), 5'-CH_2-S-CH_2-3' (sulfide), 5'-O-CH_2-O-3' (formacetal), 5'-S-CH_2-O-3' (3'-thioformacetal), 5'-O-CH_2-S-3' (5'-thioformacetal), 5'-CH_2-CH_2-S-3' (thioether), 5'-CH_2-NH-O-3' (hydroxylamine), 5'-CH_2-N(CH_3)-O-3' (methylene(methylimino)), 5'-CH_2-O-N(CH_3)-3' (methyleneoxy(methylimino)), 5'-O-C(O)-NH-3' (5'-N-carbamate), 5'-CH_2-C(O)-NH-3' (amide), 5'-NH-C(O)-CH_2-3' (amide 2), 5'-CH_2-NH-C(O)-3' (amide 3) and 5'-C(O)-NH-CH_2-3' (amide 4), and the tautomeric forms thereof;

or one of V and W is such an internucleosidic bridging group and the other is a terminal radical selected from the group consisting of -OH and $-NH_2$, preferably -OH; and

B is a radical of a nucleic acid base;

with the proviso that if Q is H, then at least one of V or W is an internucleosidic bridging group other than 5'-O-P(O)(OH)-O-3' (phosphodiester).

19. (Amended) A process for the preparation of an oligonucleotide derivative according to claim 1, said process comprising incorporating at least one building block of formula (I) according to claim 8 into the oligonucleotide derivative during oligonucleotide synthesis.

20. (Amended) A pharmaceutical composition comprising an oligonucleotide derivative according to claim 26 together with a pharmaceutically acceptable excipient and/or auxilliary substance, said pharmaceutical composition being suitable for administration to humans suffering from a disease that responds to the modulation of human bcl-xL expression or that responds to the modulation of human bcl-xL and human bcl-2 expression.

21. (Amended) An oligonucleotide derivative according to claim 1 for use in medicine.

22. (Amended) Use of an oligonucleotide derivative according to claim 1 in the preparation of a pharmaceutical composition for treatment of a disease status associated with the biosynthesis of human bcl-xL protein or with the biosynthesis of both the human bcl-xL protein and the human bcl-2 protein.

23. (Amended) A method of treatment of a disease status associated with the expression of human bcl-xL protein or with the expression of both the human bcl-xL protein and the human bcl-2 protein, comprising application of an oligonucleotide derivative according to claim 1.

24. (Amended) A method of modulating the biosynthesis of human bcl-xL protein in a cell, comprising application of an oligonucleotide derivative according to claim 1 to said cell.

25. (Amended) An oligonucleotide derivative according to claim 1 for use in a diagnostic method.

Please add claim 26 as set out below:

26. A pharmaceutical composition comprising an oligonucleotide derivative according to claim 1.

REMARKS

The foregoing amendments are made to fix multiple dependencies and correct various informalities in the claims and to place these claims in better condition for allowance. No new matter is introduced thereby. It is submitted that each of claims 1-26 are now in condition for allowance. Should the Examiner wish to discuss any issue of form or substance he or she is invited to contact the undersigned attorney at the telephone number listed below.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned **"Version with markings to show changes made."**

Respectfully submitted,
MARSHALL, GERSTEIN & BORUN

By _____

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October 30, 2001

10/070184 37 Rec'd PCT 30 OCT 2001

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the claims:

Claims 3, 4, 6, 7, 8, 19, 20, 21, 22, 23, 24, and 25 have been amended as set out below:

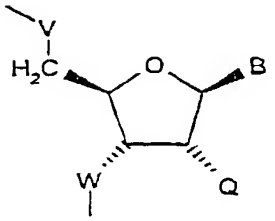
3. (Amended) The oligonucleotide derivative according to claim 1 [any of claims 1 or 2] comprising a base sequence which is complementary to at least a part of the said region of the human bcl-xL mRNA or the human bcl-2 mRNA, or wherein such base sequence contains up to 3 mispairing building blocks, or wherein such base sequence contains up to 3 abasic building blocks.

4. (Amended) The oligonucleotide derivative according to claim 1 [any of claims 1 to 3] having a length of 8 to 25 consecutive building blocks.

6. (Amended) The oligonucleotide derivative according to claim 3 [any of claims 3 to 5] wherein said base sequence is selected from the group consisting of the base sequence 5′ -AAGGCATCCCAGCCTCCGTT-3′ (SEQ. ID NO.: 3), the base sequence 5′-AAAGTATCCCAGCCGCCGTT-3′ (SEQ ID NO: 4), and the base sequence 5′-AAAGCATCCCAGCCTCCGTT-3′ (SEQ ID NO: 5).

7. (Amended) The oligonucleotide derivative according to claim 1 [any of claims 1 to 6], consisting of a base sequence selected from the group consisting of the base sequence 5′ -AAGGCATCCCAGCCTCCGTT-3′ (SEQ ID NO: 3), the base sequence 5′-AAAGTATCCCAGCCGCCGTT-3′ (SEQ ID NO: 4) and the base sequence 5′-AAAGCATCCCAGCCTCCGTT-3′ (SEQ ID NO: 5).

8. (Amended) The oligonucleotide derivative according to claim 1 [any claims 1 to 7], comprising at least one building block of formula (I)



(I),

wherein

Q is H, $-OCH_3$, $-O(CH_2CH_2)_nOCH_3$, or $-OCH_2CH_2NR_1R_2$, wherein R_1 and R_2 are, independently of each other, H or $-CH_3$, and wherein n is 1, 2 or 3;

V and W are, independently of each other, the same or different radicals of an internucleosidic bridging group selected from the following group: 5'-O-P(O)(OH)-O-3' (phosphodiester), 5'-O-P(O)(SH)-O-3' (phosphorothioate), 5'-O-P(S)(SH)-O-3' (phosphodithioate), 5'-O-P(O)(CH_3)-O-3' (methylphosphonate), 5'-O-P(O)(NH-R_7)-O-3' (phosphoamidate) in which R_7 is C_1-C_3alkyl, 5'-O-P(O)(OR_8)-O-3' (phosphotriester) in which R_8 is C_1-C_3alkyl, 5'-O-S(O)$_2$-CH_2-3' (sulfonate), 5'-O-S(O)$_2$-NH-3' (sulfamate), 5'-NH-S(O)$_2$-CH_2-3' (sulfonamide), 5'-CH_2-S(O)$_2$-CH_2-3' (sulfone), 5'-O-S(O)-O-3' (sulfite), 5'-CH_2-S(O)-CH_2-3' (sulfoxide), 5'-CH_2-S-CH_2-3' (sulfide), 5'-O-CH_2-O-3' (formacetal), 5'-S-CH_2-O-3' (3'-thioformacetal), 5'-O-CH_2-S-3' (5'-thioformacetal), 5'-CH_2-CH_2-S-3' (thioether), 5'-CH_2-NH-O-3' (hydroxylamine), 5'-CH_2-N(CH_3)-O-3' (methylene(methylimino)), 5'-CH_2-O-N(CH_3)-3' (methyleneoxy(methylimino)), 5'-O-C(O)-NH-3' (5'-N-carbamate), 5'-CH_2-C(O)-NH-3' (amide), 5'-NH-C(O)-CH_2-3' (amide 2), 5'-CH_2-NH-C(O)-3' (amide 3) and 5'-C(O)-NH-CH_2-3' (amide 4), and the tautomeric forms thereof;

or one of V and W is such an internucleosidic bridging group and the other is a terminal radical selected from the group consisting of -OH and $-NH_2$, preferably -OH; and

B is a radical of a nucleic acid base;

with the proviso that if Q is H, then at least one of V or W is an internucleosidic bridging group other than 5'-O-P(O)(OH)-O-3' (phosphodiester).

19. (Amended) A process for the preparation of an oligonucleotide derivative according to claim 1 [any claims 1 to 18], said process comprising incorporating at least one building block of formula (I) according to claim 8 into the oligonucleotide derivative during oligonucleotide synthesis.

20. (Amended) A pharmaceutical composition comprising an oligonucleotide derivative according to claim 26 [any of claims 1 to 18 optionally] together with a pharmaceutically acceptable excipient and/or auxilliary substance, said pharmaceutical composition being suitable for administration to humans suffering from a disease that responds to the modulation of human bcl-xL expression or that responds to the modulation of human bcl-xL and human bcl-2 expression.

21. (Amended) An oligonucleotide derivative according to claim 1 [any claims 1 to 18] for use in medicine.

22. (Amended) Use of an oligonucleotide derivative according to claim 1 [any of claims 1 to 18] in the preparation of a pharmaceutical composition for treatment of a disease status associated with the biosynthesis of human bcl-xL protein or with the biosynthesis of both the human bcl-xL protein and the human bcl-2 protein.

23. (Amended) A method of treatment of a disease status associated with the expression of human bcl-xL protein or with the expression of both the human bcl-xL protein and the human bcl-2 protein, comprising application of an oligonucleotide derivative according to claim 1 [any of claims 1 to 18].

24. (Amended) A method of modulating the biosynthesis of human bcl-xL protein in a cell, comprising application of an oligonucleotide derivative according to claim 1 [any of claims 1 to 18] to said cell.

25. (Amended) An oligonucleotide derivative according to claim 1 [any of claims 1 to 18] for use in a diagnostic method.

Claim 26 has been added as set out below:

26. A pharmaceutical composition comprising an oligonucleotide derivative according to claim 1.

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PATENT
Attorney Docket No.: 27656/37821

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:

ZANGEMEISTER-WITTKE et al

U.S. National Phase of PCT/EP00/03708 filed 26 April 2000

Filed: Herewith

For: **OLIGONUCLEOTIDE DERIVATIVES DIRECTED AGAINST HUMAN BCL-XL AND HUMAN BCL-2 MRNA**

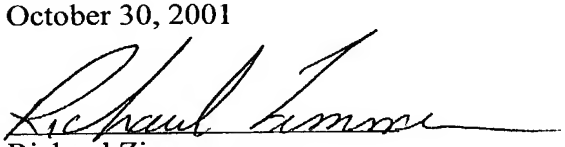
Group Art Unit: Unassigned

Examiner: Unassigned

Attorney Docket No. 27656/37821

I hereby certify that this paper is being deposited with the United States Postal Service on **October 30, 2001**, in an envelope addressed to the Commissioner for Patents, Washington, D.C. 20231 utilizing the "Express Mail Post Office to Addressee" service of the United States Postal Service under Mailing Label No. EL564 464 932US.

October 30, 2001



Richard Zimmermann

STATEMENT UNDER 37 C.F.R. §§1.821(f)

BOX PCT
Commissioner for Patents
Washington, DC 20231

Sir:

I hereby state that the content of the paper and computer readable copies of the Sequence Listing, submitted herewith in accordance with 37 C.F.R. §§1.821 and 1.825, are the same.

Respectfully submitted,

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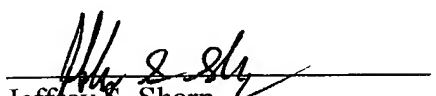
By 

Jeffrey S. Sharp
Registration No. 31,879
Attorneys for Applicants

October 30, 2001

Oligonucleotide derivatives directed against human bcl-xL and human bcl-2 mRNA.

The present invention relates to antisense oligonucleotide derivatives directed against human bcl-xL mRNA and being capable of modulating the biosynthesis of human bcl-xL protein. Furthermore, the present invention relates to antisense oligonucleotide derivatives directed against both human bcl-xL mRNA and human bcl-2 mRNA, and being capable of modulating the biosynthesis of both human bcl-xL protein and human bcl-2 protein. The present invention further relates to a pharmaceutical composition comprising such oligonucleotide derivatives, uses thereof and methods of treatment and diagnosis utilizing such oligonucleotide derivatives.

Human bcl-xL and human bcl-2 are proteins which are closely associated with the process of programmed cell death (apoptosis). Apoptosis is an active, tightly regulated physiological process involved in development, normal cell turnover, and hormone-induced tissue athropy. Lack of programmed cell death plays an important role in cancer and other hyperproliferative diseases like restenosis, fibrosis, psoriasis or certain types of allergic diseases, in particular in tumor progression and, importantly, might contribute to the clinical problem of resistance to anti-neoplastic regimens, in particular standard chemotherapeutic drugs.

Human bcl-xL and human bcl-2 have distinct patterns of tissue expression. In contrast to most normal tissues, in malignant tumors, such as a small cell lung cancer (SCLC) and non-small lung cancer (NSCLC), bcl-xL and bcl-2 are often co-expressed.

Oligonucleotides or, in particular, oligonucleotide derivatives directed against human bcl-xL mRNA or human bcl-2 mRNA may be used in an antisense technology strategy interfering with expression of bcl-xL and bcl-2, respectively.

Accordingly, there is an ongoing need for oligonucleotides or oligonucleotide derivatives showing high efficacy, preferably improved efficacy, in modulating the biosynthesis or expression of human bcl-xL and/or human bcl-2, in particular for the treatment of hyperproliferative diseases, e.g. those as mentioned above.

In the context of the present invention, surprisingly it has been identified that a specific region of the human bcl-xL mRNA encoding human bcl-xL protein, and a specific region of the human bcl-2 mRNA encoding human bcl-2 protein, are particularly accessible for hybridization with an antisense oligonucleotide derivative, in particular under physiological conditions, for example as encountered in a cellular environment. Both regions share a high degree of homology. In case of human bcl-xL, the said region encompasses nucleotide no. 687 (5') to no. 706 (3') of the human bcl-xL mRNA. In case of human bcl-2, the said region encompasses nucleotide no. 2032 (5') to 2051 (3') of human bcl-2 mRNA. In one

aspect, the present invention relates to antisense oligonucleotide derivatives directed against these regions.

Surprisingly, it has been found that the oligonucleotide derivatives according to the present invention, mentioned below, show the capability to modulate human bcl-xL biosynthesis in a cell. Moreover, some of the oligonucleotide derivatives of the present invention, mentioned below, in addition to be capable of modulating human bcl-xL biosynthesis, are able to modulate the biosynthesis of human bcl-2. The present oligonucleotide derivatives are therefore appropriate for the therapeutic treatment of diseases that respond to this modulation, especially inhibition, of bcl-xL or, in addition, bcl-2 biosynthesis. Such an inhibition may result, as one effect, in an induction of apoptosis, resulting in inhibition of cell (hyper)proliferation.

Accordingly, it is an object of the present application to provide an oligonucleotide derivative which is specifically hybridizable to a region ranging from base position no. 687 (5') to no. base position 706 (3') of the human bcl-xL mRNA encoding human bcl-xL protein.

In a preferred embodiment thereof, the oligonucleotide derivative additionally is specifically hybridizable to a region ranging from base position no. 2032 (5') to base position no. 2051 (3') of the human bcl-2 mRNA encoding human bcl-2 protein.

Within the context of the present invention, an oligonucleotide derivative is preferred which is capable of modulating the biosynthesis of the human bcl-xL protein. In a further embodiment thereof, an oligonucleotide derivative is preferred which is capable of modulating the biosynthesis of both the human bcl-xL protein and the human bcl-2 protein.

An oligonucleotide derivative according to the present invention has a length of 8 to 25 consecutive building blocks, preferably of 15 to 20 consecutive building blocks, and most preferably of 20 consecutive building blocks.

The expression "oligonucleotide derivative" is familiar to the skilled person and will only be explained here for the sake of completeness. Within the context of the present invention, the expression "oligonucleotide derivative" denotes, in particular, a derivatized oligonucleotide. An oligonucleotide per se is preferably an oligomer which consists of a sequence of natural nucleoside building blocks which are connected to each other by way of natural internucleosidic bridging groups. A natural nucleoside building block as such preferably consists of a sugar, in particular a β-D-erythropentofuranose, in particular β-D-ribose or β-D-2'-deoxyribose, together with a natural nucleic acid base which is linked to it in the β position. Within the context of the present invention, nucleic acid base preferably denotes a base which, in relation to a naturally occurring nucleoside, is capable of forming Watson-Crick base pairing (for antisense oligonucleotides). Examples of natural nucleic acid bases are adenine, guanine, cyto-

sine, thymine and uracil and also other bases with which the skilled person is familiar. In an oligonucleotide, a natural internucleosidic bridging group, in particular a phosphodiester group, connects the individual nucleosides to each other, in each case by way of the 3'- and 5'-positions. In this connection, "natural" preferably means that the corresponding compounds or residues occur in nature and are accessible by isolation from corresponding natural products or by means of chemical synthesis. Consequently, those compounds or residues which are not regarded as being natural are termed "derivatives" or "analogues". Within the context of this invention, the term "oligonucleotide derivative" therefore preferably denotes an oligonucleotide which is structurally modified, as compared with a corresponding natural oligonucleotide, at least one position of at least one building block (this can e.g. relate to the sugar or the base of a nucleoside building block, or to an internucleosidic bridging group). Thus, for example, another internucleosidic bridging group can be present in place of the naturally occurring phosphodiester bond; another linkage, for example a 2'-5' linkage, can be present in place of the natural internucleosidic 3'-5' linkage of nucleoside building blocks; a base analogue which is likewise capable of hybridizing with the strand of a target nucleic acid within the sense of Watson-Crick base pairing can be present in place of a natural base; or the sugar residue can be derivatized at different positions, for example at the 2'-position or the 6'-position. Moreover, an oligonucleotide derivative can also comprise, in place of at least one nucleoside building block, at least one nucleoside analogue which encompasses a non-sugar backbone to which a nucleic acid base is linked.

Derivatized oligonucleotides, nucleosides, internucleosidic bridging groups and analogues have been described (cf., for example, De Mesmaeker, A. et al., Curr. Op. Struct. Biol. 5 (1995), pp. 343 - 355; Sanghvi, Y.S. et al., (Ed.), "Carbohydrate Modifications in Antisense Research", ACS Symposium Series 580 (1994); S.T. Crooke, "Therapeutic Applications of Oligonucleotides", R. G. Landes Company Publisher (1995)).

Within the context of the present invention, "modulation" of the biosynthesis or expression of the human bcl-xL protein or human bcl-2 protein denotes an interference with the biosynthesis or expression, in particular a partial or complete inhibition thereof, in particular in connection with the translation or transcription process. Such an inhibition, in particular due to partial or complete degradation of the target nucleic acid, due to the process for translating the target nucleic acid being completely or partially inhibited, or due to the transcription process being completely or partially inhibited, can be determined by means of known methods, for example by means of the Northern blot technique at the level of the target nucleic acid, or by means of the Western blot technique at the protein level (cf., for example, Sambrook, J., Fritsch, E.F. and Maniatis, T.: "Molecular Cloning: A Laboratory Manual", 2nd Edition, Cold Spring Harbor Laboratory Press, 1989). For further means, reference is made to the Examples as mentioned below. In this regard, the term "hybridization" in particular denotes binding by way of hydrogen bonds, known as "Watson-Crick base-pairing", between complementary bases of an oligonucleotide derivative according to the invention, on the one hand, and of a target nucleic acid, on the other hand. Guanine and cytosine are an example of complementary bases between which three hy-

drogen bonds are formed. Adenine and thymine, or adenine and uracil, are examples of complementary bases between which two hydrogen bonds are formed in each case. "Specific hybridization" denotes that a sufficient degree of complementarity exists between the oligonucleotide derivative according to the invention and the target nucleic acid to enable specific binding between the oligonucleotide derivative and the nucleic acid to be achieved. For achieving specific hybridization, there must exist a sufficiently high degree of complementarity between the oligonucleotide derivative according to the invention and the target nucleic acid. An oligonucleotide derivative according to the invention hybridizes "specifically" with a target nucleic acid when the binding of the oligonucleotide to the target nucleic acid impairs the function of the latter and, furthermore, an adequate degree of complementarity is present in order to avoid non-specific binding of the oligonucleotide derivative according to the invention to a nucleic acid other than the target nucleic acid(s) when specific binding is required, for example under physiological conditions in association with an in-vivo application, such as a therapeutic treatment.

Specific hybridization can be determined, for example, by means of an in-vitro hybridization assay, preferably under physiological conditions, between an oligonucleotide derivative according to the invention and a target nucleic acid. Appropriate reaction conditions are known (cf., for example, Sambrook, J., Fritsch, E.F. and Maniatis, T.: "Molecular Cloning: A Laboratory Manual", 2nd Edition, Cold Spring Harbor Laboratory Press, 1989, Vol.2, 9.47 to 9.58). In this context, further reference is made to those methods as described in the Examples, below.

The term "at least a part of the said region" designates an internal part of the sequence as specified, said internal part having a length of at least 8 consecutive nucleotide building blocks.

The sequence of the human bcl-xL mRNA as referred to herein is accessible in the EMBL Nucleotide Sequence Database as HSBCLXL under accession no. Z23115. The sequence of the human bcl-2 mRNA as referred to herein is accessible in the GenBank Data Base as HUMBCL2A under accession number M13994. (The sequence of the human bcl-2 mRNA is also available in the GeneBank Data Base under accession number NM 000633. In this case said region encompasses nucleotide no. 605 (5') to no. 624 (3') of the human bcl-2 mRNA) Within the context of the present application, the numbering of nucleic acids, in particular of mRNA or corresponding cDNA sequences, relates to the respective numbering of the human bcl-xL mRNA or bcl-2 mRNA as contained in said data bases under said accession numbers. A corresponding cDNA sequence can be deduced from the mRNA sequence in particular by exchanging any base T of the cDNA sequence by a base U in the mRNA sequence, and vici versa.

Thus, the mRNA sequence ranging from nucleotide nos. 687 (5') to 706 (3') of the human bcl-xL mRNA reads as follows:
5'-AACGGCGGCUGGGAUACUUU-3' (SEQ ID NO: 1)

The mRNA sequence ranging from nucleotide nos. 2032 (5') to 2051 (3') of the human bcl-2 mRNA reads as follows:

5'-AACGGAGGCUGGGAUGCCUU-3' (SEQ ID NO: 2)

Hence, a preferred embodiment of the present invention is directed to an oligonucleotide derivative as mentioned above, comprising a base sequence which is complementary to at least a part of the said region of the human bcl-xL mRNA or the human bcl-2 mRNA, or wherein such base sequence contains up to 3 mispairing building blocks, or wherein such base sequence contains up to 3 abasic building blocks.

A further preferred embodiment thereof is directed to an oligonucleotide derivative as mentioned above, wherein said base sequence is selected from the group consisting of the base sequence 5'-AAGGCATCCCAGCCTCCGTT-3' (SEQ ID NO: 3), the base sequence 5'-AAAGTATCCCAGCCGCCGTT-3' (SEQ ID NO: 4) and the base sequence 5'-AAAGCATCCCAGCCTCCGTT-3' (SEQ ID NO: 5).

Even more preferred is an oligonucleotide derivative of the present invention, consisting of a base sequence selected from the group consisting of the base sequence 5'-AAGGCATCCCAGCCTCCGTT-3' (SEQ ID NO: 3), the base sequence 5'-AAAGTATCCCAGCCGCCGTT-3' (SEQ ID NO: 4) and the base sequence 5'-AAAGCATCCCAGCCTCCGTT-3' (SEQ ID NO: 5).

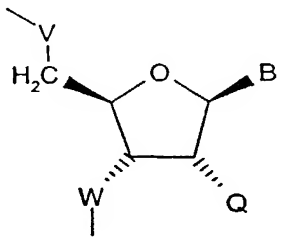
In the context of the present application the term "complementary" denotes complementarity between the base sequence of the oligonucleotide derivative and the target nucleic acid in the sense of Watson-Crick base pairing. Such complementarity can be achieved by incorporating into the oligonucleotide derivative the respective complementary base with regard to the target nucleic acid strand or a suitable analogous base. In this context, an analogous base sequence denotes a base which, although being different from the corresponding classical complementary base, upon introduction into an oligonucleotide derivative of the present invention, does not alter the oligonucleotide derivative's capability of specific hybridization with the target nucleic acid. For example cytosine can be exchanged by 5-methylcytosine, or adenine by 2-aminoadenine. Furthermore, a given base can be replaced by an inert base like hypoxanthine, as long as the function of the oligonucleotide derivative is not impaired. Such analogous or inert bases are known to the skilled person and examples for such bases are mentioned below.

Within the context of the present application the term "mispairing building block" denotes a building block of said oligonucleotide derivative which bears a nucleic acid base which is not the complementary base with respect to the respective position of the target nucleic acid strand in the sense of Watson-Crick base pairing. For example, where in the target nucleic acid strand at a specific position the

base A is located, the complementary base of the oligonucleotide derivative would be the base T, and a misparing building block can, for example, be the base C or the base G. In the same context the term "abasic building block" denotes a building block of said oligonucleotide derivative bearing no nucleic acid base capable of hybridization with the complementary base with respect to the respective position of the target nucleic acid strand in the sense of Watson-Crick base pairing. For example, an abasic building block can be a nucleoside unit solely consisting of a backbone (i.e. a sugar and an internucleosidic linkage) without comprising a nucleic acid base.

The incorporation of such a misparing or abasic building block into an oligonucleotide derivative can be tolerated as long as the resulting oligonucleotide derivative is capable of specific hybridization with the target nucleic acid and, preferably, capable of modulating the biosynthesis of human bcl-xL protein or of both the human bcl-xL and human bcl-2 proteins. Preferably, the oligonucleotide derivative according to the present invention comprises not more than up to 3 mispairing building blocks and/or not more that up to 3 abasic building blocks.

An oligonucleotide derivative according to the present invention, i.e. of any of the types as mentioned above, preferably comprises at least one building block of formula (I)



(I),

wherein

Q is H, $-OCH_3$, $-OCH_2CH_2NR_1R_2$, wherein R_1 and R_2 are, independently of each other, H or $-CH_3$, or, preferably, $-O(CH_2CH_2)_nOCH_3$, wherein n is 1, 2 or 3, preferably 1;

V and W are, independently of each other, the same or different radicals of an internucleosidic bridging group selected from the following group: 5'-O-P(O)(OH)-O-3' (phosphodiester), 5'-O-P(O)(SH)-O-3' (phosphorothioate), 5'-O-P(S)(SH)-O-3' (phosphodithioate), 5'-O-P(O)(CH_3)-O-3' (methylphosphonate), 5'-O-P(O)(NH-R_7)-O-3' (phosphoamidate) in which R_7 is C_1-C_3alkyl, 5'-O-P(O)(OR_8)-O-3' (phosphotriester) in which R_8 is C_1-C_3alkyl, 5'-O-S(O)$_2$-CH_2-3' (sulfonate), 5'-O-S(O)$_2$-NH-3' (sulfamate), 5'-NH-S(O)$_2$-CH_2-3' (sulfonamide), 5'-CH_2-S(O)$_2$-CH_2-3' (sulfone), 5'-O-S(O)-O-3' (sulfite), 5'-CH_2-S(O)-CH_2-3' (sulfoxide), 5'-CH_2-S-CH_2-3' (sulfide), 5'-O-CH_2-O-3' (formacetal), 5'-S-CH_2-O-3' (3'-thioformacetal), 5'-O-CH_2-S-3' (5'-thioformacetal), 5'-CH_2-CH_2-S-3' (thioether), 5'-CH_2-NH-O-3' (hydroxylamine), 5'-CH_2-N(CH_3)-O-3' (methylene(methylimino)), 5'-CH_2-O-N(CH_3)-3' (methyleneoxy(methylimino)),

5'-O-C(O)-NH-3' (5'-N-carbamate), 5'-CH_2-C(O)-NH-3' (amide), 5'-NH-C(O)-CH_2-3' (amide 2), 5'-CH_2-NH-C(O)-3' (amide 3) and 5'-C(O)-NH-CH_2-3' (amide 4), and the tautomeric forms thereof;

or one of V and W is such an internucleosidic bridging group and the other is a terminal radical selected from the group consisting of -OH and -NH_2, preferably -OH; and

B is a radical of a nucleic acid base;

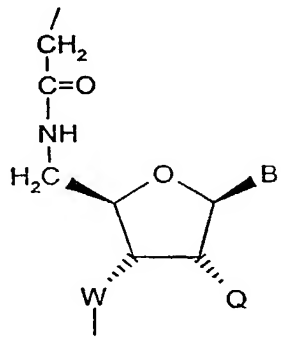
with the proviso that if Q is H, then at least one of V or W is an internucleosidic bridging group other than 5'-O-P(O)(OH)-O-3' (phosphodiester).

Within the context of the present invention, Q is preferably -$OCH_2CH_2NR_1R_2$, wherein one of R_1 and R_2 is H and the other is -CH_3, or each of R_1 and R_2 is H, or, more preferably, Q is -$OCH_2CH_2OCH_3$.

Oligonucleotide derivatives according to the present invention which comprise a building block of formula (I), wherein Q is other than H, can be prepared in accordance with the methods as described, for example, by P. Martin, Helv. Chem. Acta, 78 (1995), pp. 486 - 504, or in an analogous way.

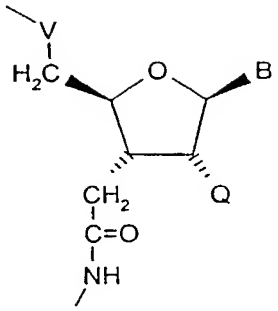
The 5' and 3' orientation of the said radicals V and W, as an internucleosidic bridging bond, in the above-mentioned formula (I), may be clarified as follows:

When V for example is a radical 5'-CH_2-C(O)-NH-3' (amide), the corresponding nucleoside building block of the above-defined formula (I) has the following structure (I.1):



(I.1).

When W for example is a radical 5'-CH_2-C(O)-NH-3' (amide), the corresponding nucleoside building block of the above-defined formula (I) has the following structure (I.2):



(I.2).

Some of the said radicals of internucleosidic bridging groups can exist in different tautomeric forms, depending, inter alia, on the solvent and on the degree of ionization of ionizable groups. Thus, for example, the bridging group in a phosphorothioate [O-(P-SH)(=O)-O] can be tautomerized to [O-(P-OH)(=S)-O], with the more stable form depending, inter alia, on the solvent and the ionization state. Within the context of the present invention, the term "oligonucleotide derivative" also encompasses those tautomeric forms which are familiar to the skilled person.

In general, within the context of the present application reference to a particular formula includes all definitions and preferences as stated for that formula.

Particularly preferably, V and W, as the radical of an internucleosidic bridging group, are selected, independently, from the following group: 5'-O-P(O)(OH)-O-3' (phosphodiester), 5'-O-P(O)(SH)-O-3' (phosphorothioate) and 5'-CH_2-C(O)-NH-3' (amide).

In particular, one of the radicals V or W, as the radical of an internucleosidic bridging group, is 5'-O-P(O)(OH)-O-3' (phosphodiester) and the other radical is 5'-O-P(O)(SH)-O-3' (phosphorothioate).

V and W are also preferably, as the radical of an internucleosidic bridging group, in each case 5'-O-P(O)(OH)-O-3' (phosphodiester) or, mostly preferred, in each case 5'-O-P(O)(SH)-O-3' (phosphorothioate).

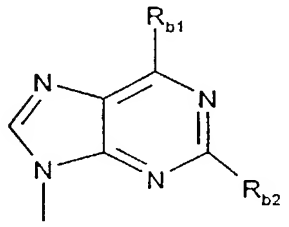
Preferred is an oligonucleotide derivative according to the present invention, comprising only building blocks of formula (I), wherein V and W each are phosphorothioate and Q is H.

Within the context of the present invention, a nucleic acid base, in particular a nucleic acid base B of formula (I), is understood as being, in particular, natural nucleic acid bases and known analogues (cf., for example, Accounts of Chem. Res. 28 (1955), pp. 366-374; Sanghvi, Y.S. in: Antisense Research and Applications, Crooke, S.T. and Lebleu, B. (Ed.), CRC Press, Boca Raton (1993), pp. 273-288) As is familiar to the skilled person, nucleic acid bases B can exist in tautomeric forms depending on the

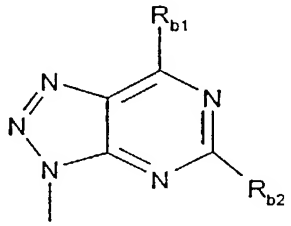
ambient conditions. According to the invention, such tautomeric forms are also encompassed by the oligonucleotide derivatives according to the invention, including the preferred embodiments.

The invention preferably relates to an oligonucleotide derivative according to the invention, including the said preferences, in which

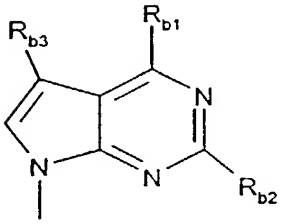
said nucleic acid base, in particular said nucleic acid base B of formula (I), is a radical of the formula (V1), (V2), (V3), (V4) or (V5)



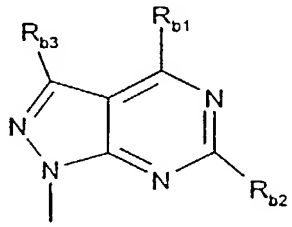
(V1),



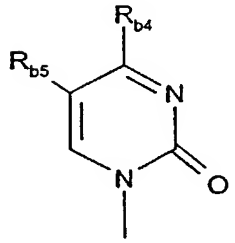
(V2),



(V3),



(V4),



(V5)

in which

R_{b1} is $-NH_2$, -SH or -OH;

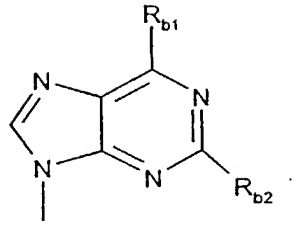
R_{b2} is H, $-NH_2$ or -OH; and

R_{b3} is H, Br, I, -CN, $-C\equiv C-CH_3$, $-C(O)NH_2$ or $-CH_3$;

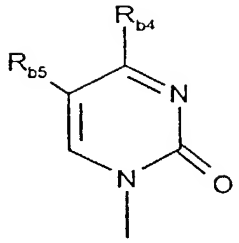
R_{b4} is $-NH_2$ or -OH; and

R_{b5} is H, F, Br, I, -CN, $-C\equiv C-CH_3$, $-C(O)NH_2$ or $-CH_3$.

In a preferred embodiment thereof, said nucleic acid base, in particular said nucleic acid base B of formula (I), is a radical of the formula (V1) or (V5)



(V1),



(V5)

in which

R_{b1} is $-NH_2$, -SH or -OH;

R_{b2} is H, $-NH_2$ or -OH;

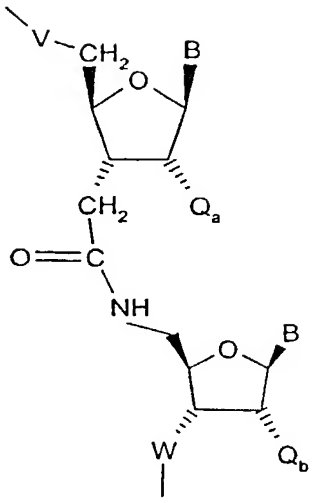
R_{b4} is $-NH_2$ or -OH; and

R_{b5} is H, F, Br, I, -CN, $-C\equiv C-CH_3$, $-C(O)NH_2$ or $-CH_3$.

Particularly preferably, said nucleic acid base, in particular said nucleic acid base B of formula (I), is selected from the group of the following radicals: xanthine, hypoxanthine, adenine, 2-aminoadenine, guanine, 6-thioguanine, uracil, thymine, cytosine, 5-methylcytosine, 5-propynyluracil, 5-fluorouracil and 5-propynylcytosine.

Preferred are oligonucleotide derivatives which (in their nucleotide/nucleotide derivative sequence) comprise at least one building block of formula (I), wherein B and Q are as defined herein, including the respective preferences and embodiments, and V and W are selected from the group consisting of the following radicals: 5'-O-P(O)(OH)-O-3' (phosphodiester), 5'-O-P(O)(SH)-O-3' (phosphorothioate) and 5'-CH_2-C(O)-NH-3' (amide),

In case at least one of V or W is 5'-CH_2-C(O)-NH-3' (amide), the oligonucleotide derivative comprises preferably the following dimeric unit (bivalent radicals) of the formula (II)



(II)

wherein Q_a and Q_b , independently of each other, are H, $-OCH_3$ or $-OCH_2CH_2OCH_3$, or preferably Q_a is $-OCH_3$ and Q_b is H, or more preferably, Q_a and Q_b are each H;

and B, V and W are as defined herein, inclusive of the respective preferences and embodiments.

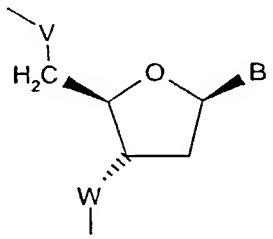
Mostly preferred is an oligonucleotide derivative according to the present invention, consisting of a base sequence selected from the group consisting of the base sequence 5'-AAGGCATCCCAGCCTCCGTT-3' (SEQ ID NO: 3) and the base sequence 5'-AAAGTATCCCAGCCGCCGTT-3' (SEQ ID NO: 4), wherein each V and each W as radicals of an internucleosidic bridging group of the building blocks according to formula (I) are of the 5'-O-P(O)(SH)-O-3' (phosphorothioate) type and wherein each Q according to formula (I) is -H.

Preference is furthermore given to an oligonucleotide derivative according to the present invention which has a "chimeric" structure. Within the context of the present invention, a "chimeric structure", also termed a "chimera", is to be understood as meaning an oligonucleotide derivative which contains 2 or more chemically different regions which are in each case synthesized from one type of nucleic acid building block. Such chimeric oligonucleotide derivatives typically comprise at least one region of modified nucleic acid building blocks which confer one or more advantageous property/properties (for example increased resistance to nucleases, increased binding affinity or diminished occurrence of sequence-independent side-effects) on the oligonucleotide derivative, the so-called "wing", also designated the M region in that which follows, and a region which enables RNAse H-mediated cleavage of the target nucleic acid to take place, i.e. the so-called "RNAse H window", also designated the U region in that which follows. The affinity of an oligonucleotide or an oligonucleotide derivative is customarily determined by measuring the T_m value of the oligonucleotide (derivative)/target nucleic acid hybrid. The T_m value is the temperature at which the oligonucleotide, or its derivative, and the target nucleic acid

dissociate from a previously formed hybrid. The dissociation is determined spectrophotometrically. The higher the T_m value, the higher is the affinity of the oligonucleotide, or the derivative, for the target nucleic acid. Methods for determining the T_m value belong to the state of the art (cf., for example, Fritsch and Maniatis, "Molecular Cloning -A Laboratory Manual", 2nd Edition, Cold Spring Harbor Laboratory Press, 1989). Within the context of the present invention, increased resistance to nucleases denotes decreased or slowed-down degradation of the oligonucleotide derivatives according to the invention by exonucleases or endonucleases which are present in a cell. The resistance to nucleases or the degradation of an oligonucleotide or a derivative can be monitored by gel electrophoresis, for example. RNAse H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of the enzyme therefore leads to cleavage of the target RNA and consequently increases the efficacy of the antisense mechanism. Cleavage of the target RNA can customarily be demonstrated by gel electrophoresis. Since, in a chimera, different advantageous properties are present in one and the same molecule, oligonucleotide derivatives according to the invention possess a pronounced antisense effect with regard to inhibiting the expression of a protein or RNA.

In one embodiment, a chimeric oligonucleotide derivative according to the present invention comprises at least one M region, which consists of at least one nucleic acid building block of the formula (I) as mentioned above, and at least one U region, which enables RNAse H-mediated cleavage of the target nucleic acid to take place. The U region consists, in particular, of customary 2'-deoxyribonucleic acid building blocks which are linked to each other by way of phosphodiester bonds, or preferably phosphorothioate bonds, as the internucleosidic group. The M region of a chimeric oligonucleotide derivative according to the present invention consists, in particular, of nucleic acid building blocks of the formula (I) in which Q is as defined, preferably $-OCH_2CH_2OCH_3$, in which W and V, as the radical of an internucleosidic bridging group, are a phosphodiester bond, a phosphorothioate bond or an amide bond, with a phosphodiester bond being preferred.

Chimeric oligonucleotide derivatives according to the invention of the above-mentioned type, which preferably consist of a total of 8 to 25, in particular of 8 to 20, and preferably of 20 consecutive building blocks, preferably of the nucleoside type, and preferably comprise one or more, preferably one, U region(s) having, preferably 4 to 13, for example 10 or 13, nucleoside building blocks of formula III



(III),

wherein B is as defined above, including the respective preferences and specific embodiments, and wherein V and W in each case are a phosphodiester group or phosphorothioate group, preferably a phosphorothioate group, as the radical of an internucleosidic bridging group,

and further comprise one or more, preferably one or two, M region(s) comprising the remaining nucleoside building blocks, which are of formula (I), in which V and W are, as the radical of an internucleosidic bridging group, in particular, in each case, a phosphodiester, phosphorothioate or amide group, preferably phosphodiester or phosphorothioate, in particular phosphodiester, and in which Q and B are as defined above, including the respective preferences and specific embodiments, Q being in particular -O-$CH_2CH_2OCH_3$.

The M and U regions in chimeric oligonucleotide derivatives according to the invention are preferably present in one of the following arrangements:

5'-M--U--M-3'
5'-M--U-3' or
5'-U--M-3'.

Additional oligonucleotide derivatives according to the invention are conjugated with other units, for example a micelle-forming group, an antibody, a carbohydrate, a receptor-binding group, a steroid such as cholesterol, a polypeptide, an intercalating agent, such as an acridine derivative, a long-chain alcohol, a dendrimer, a phospholipid and other lipophilic groups. Conjugating in this way confers advantageous properties with regard to the pharmacokinetic characteristics on the oligonucleotide derivative according to the invention. In particular, conjugating in this way achieves increased cellular uptake.

In a very particularly preferred embodiment, an oligonucleotide derivative according to the present invention consists exclusively of nucleoside building blocks of the formula (I) which are connected to each other by way of phosphodiester bonds as the internucleosidic bridging groups V and/or W. In another very particularly preferred embodiment, an oligonucleotide derivative according to the invention exclusively comprises nucleoside building blocks of the formula III which are connected to each other by way of phosphorothioate bonds as the internucleosidic bridging groups V and/or W.

Accordingly, the present invention furthermore relates to an oligonucleotide derivative as mentioned herein, consisting of a base sequence selected from the group consisting of the base sequence 5'-AAGGCATCCCAGCCTCCGTT-3' (SEQ ID NO: 3), the base sequence 5'-AAAGTATCCCAGCCGCCGTT-3' (SEQ ID NO: 4), and the base sequence 5'-AAAGCATCCCAGCCTCCGTT-3' (SEQ ID NO: 5), wherein each V and each W as radicals of an internucleosidic bridging group of all building blocks according to formula (I) are of the 5'-O-P(O)(SH)-O-3' (phosphorothioate) type, and wherein each Q according to formula (I) of the nucleotides being

underlined is $-OCH_2CH_2OCH_3$ and wherein each Q according to formula (I) of the remaining nucleotides is -H.

Provided that salt-forming groups are present, the term "oligonucleotide derivative" also encompasses salts, in particular acid addition salts, salts with bases or, if several salt-forming groups are present, possibly also mixed salts or internal salts.

Salts of oligonucleotide derivatives according to the invention are, in particular, pharmaceutically tolerated salts, i.e. essentially non-toxic salts.

Such salts are formed, for example, from the oligonucleotide derivatives according to the invention which possess an acidic group, for example a carboxyl group, a phosphodiester group or a phosphorothioate group, and are, for example, salts with suitable bases. These salts include, for example, non-toxic metal salts which are derived from metals of groups Ia, Ib, IIa and IIb of the Periodic System of the elements, in particular suitable alkali metal salts, for example lithium, sodium or potassium salts, or alkaline earth metal salts, for example magnesium or calcium salts. They furthermore include zinc and ammonium salts and also salts which are formed with suitable organic amines, such as unsubstituted or hydroxyl-substituted mono-, di- or tri-alkylamines, in particular mono-, di- or tri-alkylamines, or with quaternary ammonium compounds, for example with N-methyl-N-ethylamine, diethylamine, triethylamine, mono-, bis- or tris-(2-hydroxy-lower alkyl)amines, such as mono-, bis- or tris-(2-hydroxyethyl)amine, 2-hydroxy-tert-butylamine or tris(hydroxymethyl)methylamine, N,N-di-lower alkyl-N-(hydroxy-lower alkyl)amines, such as N,N-dimethyl-N-(2-hydroxyethyl)amine or tri-(2-hydroxyethyl)amine, or N-methyl-D-glucamine, or quaternary ammonium compounds such as tetrabutylammonium salts.

Lithium salts, sodium salts, magnesium salts, zinc salts or potassium salts are preferred, with sodium salts being particularly preferred.

Oligonucleotide derivatives according to the invention which possess a basic group, for example an amino group or imino group, can form acid addition salts, for example with inorganic acids, for example with a hydrohalic acid, such as hydrochloric acid, sulfuric acid or phosphoric acid, or with organic carboxylic acids, sulfonic acids, sulfo acids or phospho acids or N-substituted sulfamic acid, for example acetic acid, propionic acid, glycolic acid, succinic acid, maleic acid, hydroxymaleic acid, methylmaleic acid, fumaric acid, malic acid, tartaric acid, gluconic acid, glucaric acid, glucuronic acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, salicylic acid, 4-aminosalicylic acid, 2-phenoxybenzoic acid, 2-acetoxybenzoic acid, embonic acid, nicotonic acid or isonicotonic acid, and, in addition, with amino acids, for example with α-amino acids, and also with methanesulfonic acid, ethanesulfonic acid, 2-hydroxymethanesulfonic acid, ethane-1,2-disulfonic acid, benzenedisulfonic acid, 4-methylbenzenesulfonic acid, naphthalene-2-sulfonic acid, 2- or 3-phosphoglycerate, glucose-6-phosphate or N-cyclo-

hexylsulfamic acid (with formation of the cyclamates) or with other acidic organic compounds, such as ascorbic acid.

Oligonucleotide derivatives according to the invention which possess both acidic and basic groups can also form internal salts.

Oligonucleotide conjugates according to the invention which possess more than one group which is suitable for salt formation can also form mixed salts.

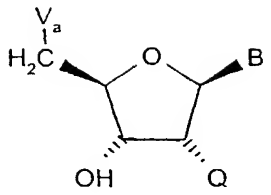
Pharmaceutically unsuitable salts, for example picrates or perchlorates, can also be used for isolation and purification.

It is only the pharmaceutically tolerated salts, which are non-toxic when used correctly, which are employed for therapeutic purposes and which are therefore preferred.

In a further embodiment the present invention relates to a process for preparing an oligonucleotide derivative according to the present invention, said process comprising incorporating at least one building block of formula (I) as mentioned above into the oligonucleotide derivative during oligonucleotide synthesis.

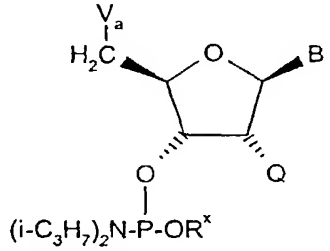
The compounds of the formula (I), wherein V and W each are a terminal group as defined above, are employed as nucleoside building blocks in the synthesis of the oligonucleotide derivatives according to the invention. The oligonucleotide derivatives according to the invention can be prepared, in a manner known per se, in accordance with a variety of methods, in DNA synthesis equipment which can be automated and which can be obtained commercially in conjunction with method protocols. For example, in the case of a phosphodiester group as the internucleosidic bridging group, the phosphotriester method, the phosphite triester method or the H-phosphonate method, which are familiar to the skilled person, can be used (cf., for example, Eckstein, F., "Oligonucleotides and Analogues, A Practical Approach", IRL Press (1991)).

In the case of the phosphite triester method, the approach can, for example, be to react, for example, a nucleoside building block of the formula (I), in which V_a and W_a are in each case -OH, with a protecting group reagent, for example 4,4'-dimethoxytriphenylmethyl chloride, to give a nucleoside of the formula (Ib)



(Ib),

in which V_a is a protected hydroxyl group and Q and B are defined as above for the compound of the formula I including the said preferences, reactive groups thereof being protected by a suitable protective group where necessary, and to bind the compound of the formula Ib with the aid of a linker, for example succinic anhydride, to a solid support material, for example to "Controlled Pore Glass" (CPG), which contains long-chain alkylamino groups. In a separate procedure, the hydroxyl group of another nucleoside building block of the formula (Ib) is derivatized, for example using R^xO-P[N(i-propyl)$_2$)]$_2$ to give a phosphoramidite of the formula (Ic)



(Ic),

in which R^x is a customary protecting group, for example ß-cyanoethyl, wherein B and Q are defined above.

Protecting groups for the radical V_a as protected hydroxyl group in compounds of the formulas (Ib) and (Ic) preferably are of the trityl-type, in particular trityl (Tr), 4-monomethoxytrityl (MMTr), preferably 4,4'-dimethoxytrityl (DMTr) and, likewise preferably, 4,4',4"-tris-tert-butyltrityl (TTTr).

After the protecting group on the radical V_a, for example the DMTr- or the TTTr group, of the support-bound material has been eliminated, this material is coupled, with elimination of -N(i-C_3H_7)$_2$ to the compound of the formula (Ic), any free hydroxyl groups which may be present are blocked ("capping") and the phosphite which has been formed is then oxidized, thereby leading, for example, to the phosphate or phosphorothioate. After the dimer has been deprotected, the reaction cycle is repeated with a compound of the formula (Ic) until an oligomer having the desired number of monomer units has been synthesized, and the product is then detached from the support material. In this way, an oligonucleotide derivative according to the invention is obtained which is synthesized entirely from nucleoside building blocks of the formula (I), above, having phosphodiester groups or phosphorothioate groups, depending on the oxidation conditions, as the internucleosidic bridging group. Depending on the use of

appropriate nucleoside building blocks in the individual reaction cycles, oligonucleotides according to the invention of any arbitrary sequence can be prepared in an analogous manner, in particular those oligonucleotides according to the invention which, in addition to one or more nucleoside building block(s) of the formula (I), contain other nucleoside building blocks, in particular those comprising structures of formulas (II) and/or (III).

Oligonucleotide derivatives according to the present invention which do not contain, or which do not exclusively contain, phosphodiester groups or phosphorothioate groups as the internucleosidic bridging groups can be prepared in a manner known per se (cf., for example, the above-mentioned publications of De Mesmaeker, A., or Crooke, S.T.).

The oligonucleotide derivatives according to the invention possess a number of advantageous properties. These include, in particular, a high binding affinity for a target nucleic acid and a high resistance to nucleases. Furthermore, they are capable of a sequence-specific effect, are taken up satisfactorily by a cell and have adequate bioavailability. These properties make the oligonucleotides according to the invention particularly suitable for pharmaceutical applications, in particular for modulating the biosynthesis or expression of the human bcl-xL protein or both of the human bcl-xL and bcl-2 proteins. Consequently, in a further aspect the present application relates to an oligonucleotide derivative according to the present invention for use in medicine.

An oligonucleotide derivative according to the invention can be used, in particular, as an antisense oligonucleotide. The expression "antisense" is known to the skilled person and, in the context of the present invention, characterizes, in particular, the relationship between an oligonucleotide derivative according to the invention and the sequence, which is complementary to it, of a target nucleic acid (i.e. human bcl-xl mRNA or bcl-2 mRNA), namely that the oligonucleotide derivative and the complementary sequence are able to hybridize to each other. The identification of a suitable antisense oligonucleotide is a multi-step process. First of all, a target nucleic acid is identified which underlies the protein whose expression characterizes a pathological state in humans, and is to be modulated. In the present case, the target nucleic acid is, in particular, the RNA which is transcribed from the gene which encodes the protein of interest, such as pre-mRNA or, preferably, the (mature) mRNA. Within the target nucleic acid, a sequence or sequences is/are identified which interact, in particular by means of hybridization, with the oligonucleotide derivative according to the invention such that expression of the protein of interest is modulated. An oligonucleotide derivative according to the invention must possess a complementarity to the target nucleic acid which is adequate, due to sufficiently powerful and sufficiently specific hybridization, to achieve the desired effect.

Consequently, the invention also provides the use of an oligonucleotide derivative according to the invention, including the said preferences, as an antisense oligonucleotide.

According to the invention, oligonucleotide derivatives are preferred which are capable of modulating the expression (biosynthesis) of the human bcl-xL protein. Even more preferred are oligonucleotide derivatives which are capable of modulating the expression (biosynthesis) of both the human bcl-xL protein and the human bcl-2 protein.

The invention furthermore relates to a pharmaceutical composition which comprises an oligonucleotide derivative according to the invention, or a pharmaceutically tolerated salt thereof, in a pharmaceutically effective quantity, if desired together with a pharmaceutically tolerated excipient and/or auxiliary substance. Such a pharmaceutical composition preferably is suitable for administration to humans suffering from a disease that responds to the modulation of human bcl-xL expression or that responds to the modulation of human bcl-xl and human bcl-2 expression.

Pharmaceutical compositions according to the invention (and also oligonucleotide derivatives according to the invention) can be used, for example, for the therapeutic or prophylactic treatment of hyperplastic or neoplastic states, for example of cancer, in particular solid tumors, or of restenosis, fibrosis or psoriasis, preferably in a cancer selected from the group consisting of colorectal cancer, gastric cancer, prostate cancer, neuroblastoma, melanoma, thyroid cancer, renal cancer, breast cancer and, preferably, lung cancer, the letter being, for example, in particular NSCLC (non-small cell lung cancer) or, most preferably, SCLC (small cell lung cancer).

In particular, pharmaceutical compositions according to the invention (and also oligonucleotide derivatives according to the invention) are capable of killing cancer cells, preferably by induction of apoptosis, and/or of reverting multidrug resistance of tumors.

Pharmaceutical compositions which are preferred in accordance with the invention comprise preferred oligonucleotide derivatives as described above.

The pharmaceutical compositions according to the invention are preferably present in the form of preparations which can be administered parenterally or of infusion solutions. Aqueous solutions of the active substance in water-soluble form, for example in the form of one of the above-mentioned water-soluble salts, in the presence or absence of salts, such as NaCl, and/or pharmaceutically tolerated excipient materials, such as sugar alcohols, for example mannitol, are suitable, in particular, for parenteral administration, for example for intravenous or intraperitoneal administration. Aqueous suspensions for injection which comprise viscosity-increasing substances, such as sodium carboxymethyl cellulose, sorbitol and/or dextran, are also suitable for parenteral administration. These preparations or solutions are preferably isotonic aqueous solutions or suspensions. The active substance can be present, for example, in the form of a lyophilisate, if necessary together with a pharmaceutically tolerated excipient material, which lyophilisate is brought into solution, before its use for parenteral administration, by adding a suitable solvent. These solutions which are suitable for parenteral administration can

also be employed as infusion solutions. The pharmaceutical compositions according to the invention can be sterilized and/or comprise auxiliary substances, for example preservatives, stabilizers, wetting agents and/or emulsifying agents, solubilizing agents, salts for regulating the osmotic pressure and/or buffers.

The pharmaceutical preparations, which, if desired, can comprise additional pharmacologically (or pharmaceutically) active compounds, for example antibiotics, are prepared in a manner known per se, for example by means of conventional solubilizing or lyophilizing methods, and comprise from about 0.0001 % by weight to about 95 % by weight, preferably from about 0.1 % by weight, to about 90 % by weight, in particular from about 0.5 % by weight to about 30 % by weight, for example from 1 % by weight to 5 % by weight, of active compound(s). Dosage forms in the form of individual doses comprise, for example, from about 0.001 % by weight to about 20 % by weight, of active compound(s); dosage forms which are not in the form of individual doses comprise, for example, from about 0.001 % by weight to about 10 % by weight of active compound(s). Dose units preferably comprise from about 0.0005 mg to about 0.5 mg, preferably from about 0.005 mg to about 40 mg of active compound(s), depending on the nature of the mammalian subject, including man, to be treated, on the disease to be treated and on the condition of the patient, in particular its/his/her body weight, its/his/her age and its/his/her individual state of health, and also on individual pharmacokinetics contributing factors and the route of administration.

In order to improve activity, the pharmaceutical compositions according to the invention can comprise cationic lipids.

Pharmaceutical compositions according to the invention are also preferred which additionally comprise a customary cytostatic agent. Such combination preparations are preferably employed for treating hyperplastic or neoplastic states such as cancer.

The present invention furthermore relates to an oligonucleotide derivative according to the invention, including the above-mentioned preferences, or a pharmaceutically tolerated salt thereof, for use in the prophylactic or therapeutic treatment of humans, in particular of a pathological state, which is characterized by the expression or biosynthesis of human bcl-xL protein or of both human bcl-xL and human bcl-2 proteins.

The present invention furthermore relates to the use of an oligonucleotide derivative according to the invention, including the above-mentioned preferences, for preparing a pharmaceutical composition for the prophylactic or therapeutic treatment of a pathological state in humans, which is characterized by the expression or biosynthesis of human bcl-xL protein of both human bcl-xL and human bcl-2 proteins.

Over and above this, the present invention relates to a method for the prophylactic or therapeutic treatment of a pathological state in a mammalian subject, including man, which state is characterized by the expression or biosynthesis of human Bcl-xL protein or of both human bcl-xL and human bcl-2 proteins, which method comprises administering a pharmaceutical composition according to the invention to man.

Moreover, the invention relates to a method for modulating the expression of human Bcl-xl protein or of both human bcl-xL and human bcl-2 proteins in a cell, which comprises bringing the cell, or a tissue or body fluid which contains this cell, into contact with an oligonucleotide derivative according to the invention, including the above-mentioned preferences, or with a pharmaceutical composition according to the invention. Such a process for modulating the expression or biosynthesis of a protein in a cell can be advantageously applied both in vitro and in vivo.

The oligonucleotide derivatives according to the invention, including the above-mentioned preferences, are also suitable for use as diagnostic agents and can be employed, for example, in a manner known per se, as gene probes for detecting genetically determined diseases or viral infections by means of selective interaction at the level of single-stranded or double-stranded target nucleic acids. In particular, a diagnostic application is possible in vivo as well as in vitro, due to the increased stability towards nucleases. The diagnosis can take place, for example, on isolated tissue samples, blood plasma, blood serum or other body fluids, and, in the case of in-vivo diagnosis, on tissues, cells or body fluids in the patient to be investigated as well. In a preferred embodiment thereof, such diagnosis takes place under physiological conditions.

Another aspect of the present invention consequently relates to an oligonucleotide derivative according to the invention, including the above-mentioned preferences, for use in a diagnostic method. As mentioned above, the oligonucleotide derivatives according to the invention are suitable both for in-vivo and for in-vitro diagnostic methods.

It is to note that the entire content of the references, patents and publications cited in this application is hereby incorporated by reference.

The following examples clarify the invention but do not restrict it. Examples are in particular directed to preferred embodiments of the present invention.

Examples

Example 1: Cell Culture

The SW2 SCLC cell line is obtained from the Dana Farber Cancer Institute, Boston, MA and The NSCLC cell line NCI-H125 cell line is obtained from the American Type Tissue Culture Collection (ATCC; Rockville, MD). Lung cancer cell lines are cultured in RPMI-1640 medium (Gibco Life Technologies Ltd, Paisley, UK) supplemented with 2 mM L-glutamine, 10% fetal calf serum (FCS Hy-Clone II Fetal Clone II), 50 IU/mL penicillin and 50 µg/mL streptomycin at 37°C in a humidified atmosphere with 5% CO_2. SW2 cells are grown as floating aggregates, H125 cells are grown as monolayers.

Example 2: Synthesis of oligonucleotide derivatives

The oligonucleotides of the present invention can be prepared according to methods known in the art.

For 1 µmole scale syntheses, a Perseptive Expedite MOSS synthesizer is used. The syntheses are performed on Polystyrene Primer support (Pharmacia) loaded with the 3'-end residue. Solutions at 0.05M in Acetonitrile of ß-cyanoethyl deoxyribonucleosides phosphoramidites (Amersham Pharmacia Biotech) or ß-cyanoethyl 2'-methoxyethylribonucleosides phosphoramidites (P. Martin, Helvetica Chimica Acta, 78 (1995), 486 - 504) are used. In the coupling step, phosphoramidites are activated by Benzimidazolium Triflate (0.2M in Acetonitrile; R. Noyori, J.Org. Chem. 61, 1996, 7996 - 7997). Sulfurization can be performed by a 0.5 M solution of phenyl acetyl disulfide or PADS in acetonitrile/picoline (3:1) as described (van Boom et al., Tet. Letters 1989, 30(48), pp. 6757-6760). Capping and washing steps are carried out by standard reagents and solvents. The synthetic cycle is depicted in table 1.

Table 1: Synthetic cycle for 1 µmole

Step	Reagent/Solvent	Function	Time in sec. per cycle (repeat)
1	MeCN	Wash	20 (2x)
2	3% CCl_3COOH/CH_2Cl_2	Detritylation	20+40
3	MeCN	Wash	20 (2x)
4	Nucleotide/activator/MeCN	Coupling	180
5	MeCN	Wash	20 (2x)
6	PADS 0.5M in acetonitrile/picoline 3:1	Sulfurization/Oxidation	20 (2x)
7	MeCN	Wash	20 (2x)
8	Ac_2O/lutidine/NMI/THF	Capping	10+20
9	MeCN	Wash	20 (2x)

Synthesis at larger scales (synthesis scale of 150 µmole or more) are on the Oligopilot II (Amersham Pharmacia Biotech) DNA synthesizer performed according the cycle described in table 2 with the same solutions except for the phosphoramidites which are 0.2 M in Acetonitrile.

Table 2:

Step	Reagent/Solvent	Function	Volume or equiv.
1	MeCN	Wash	6 CV
2	3% CCl_3COOH/CH_2Cl_2	Detritylation	6 CV
3	MeCN	Wash	6 CV
4	Nucleotide/activator/MeCN	Coupling	1.5 equiv
5	MeCN	Wash	6 CV
6	PADS 0.5M in acetontrile/picoline 3:1	Sulfurization	2 CV
7	MeCN	Wash	6 CV
8	Ac_2O/lutidine/NMI/THF	Capping	0.5 CV; 0.5 min.
9	MeCN	Wash	6 CV

CV is column volume

Crude product of approximately 70 % purity is further purified by reverse phase chromatography using C18 stationary phases. The purified material is lyophilized, redissolved in water, quantified by UV absorption at 260 nm and checked using standard procedures by capillary gel electrophoresis for purity and by MALDI-TOF MS for identification. For larger scale oligonucleotides, the purified material is redissolved in 100 mM NaCl and further desalted by ultrafiltration. The oligonucleotides are finally lyophilized and stored as dry materials.

The following oligonucleotide derivatives (OD) are synthesized, all being 20-mer phosphorothioates purified by high pressure liquid chromatography (HPLC). The sequences are

OD1: 5'-AAGGCATCCCAGCCTCCGTT-3' (SEQ ID NO: 3)
OD2: 5'-AAAGTATCCCAGCCGCCGTT-3' (SEQ ID NO: 4)
OD3: 5'-AAGGCATCCCAGCCTCCGTT-3' (SEQ ID NO: 3)
OD4: 5'-AAAGTATCCCAGCCGCCGTT-3' (SEQ ID NO: 4)
OD5: 5'-AAAGCATCCCAGCCTCCGTT-3' (SEQ ID NO: 5)

The underlined nucleotides of OD3, OD4 and OD5 denote building blocks bearing a 2'-methoxyethoxy substituent.

These oligonucleotides OD1, OD2, OD3, OD4 and OD5 are the most preferred embodiment of the present invention.

The following oligonucleotide derivative CO1 for use as scrambled control oligonucleotide derivative to OD2 is likewise synthesised:

CO1: 5'-CCATGCTATCGCCTACACGG-3' (SEQ ID NO: 6)

The following oligonucleotide derivative CO2 targets the translation initiation site of the bcl-2 mRNA and is used as a bcl-2 specific control:

CO2: 5'-CCAGCGTGCGCCATCCTTCC-3' (SEQ ID NO: 7)

The following oligonucleotide derivatives CO3 and CO4 are synthesized in the same manner. CO3 and CO4 are used as scrambled controls to OD3 and OD4, respectively.

CO3: 5'-CACGTCACGCGCGCACTATT-3' (SEQ ID NO: 8)
CO4: 5'-CATATCACGCGCGCACTATG-3' (SEQ ID NO: 9)

The underlined nucleotides of C3 and C4 designate building blocks bearing a 2'-methoxyethoxy substituent.

Example 3: Delivery of oligonucleotide derivatives to tumor cells

Antisense oligonucleotide derivatives OD1 and OD2 and the CO1 control oligonucleotide derivative are delivered to cells in the form of complexes with the cationic lipid N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methylsulfate (DOTAP) (Boehringer Mannheim, Germany) under serum free conditions. Equal volumes of ODNs (6 µM) and DOTAP (0.2 mM) in HEPES-buffered saline are mixed and allowed to complex for 10 min at room temperature. Different dilutions of the stock solutions are prepared in RPMI-1640 depending on the experiment, and the mixtures are further diluted into 9 volumes of cell suspension to achieve a final density of 2×10^5 cells/ml in the cultures. After 4 h of incubation serum is added to the cultures to a final concentration of 10%. Control cultures ware treated with equivalent concentrations of DOTAP or medium alone (untreated). Cells are incubated at 37°C under standard cell culture conditions for different time periods depending on the experiment.

Example 4: Northern blot analysis

To examine the effect of the antisense oligonucleotide derivatives to downregulate bcl-2 and bcl-xL expression on the transcriptional level, Northern blot analyses of SW2 cells is performed after treat-

ment with OD1 or OD2, respectively, at a concentration of 150 nM. In this study the SW2 cell line is used for the molecular analyses of the antisense effects because it expresses nearly equivalent amounts of both death antagonists bcl-2 and bcl-xL. The antisense oligonucleotide derivative CO2 that targets the translation initiation site of the bcl-2 mRNA is used as a bcl-2-specific control.

Total RNA is prepared from 5 x 10^6 SW2 cells by use of the RNeasy Mini Kit (Qiagen GmbH, Hilden, Germany) and QIAshredder (Quiagen GmbH) for homogenization. For each sample, 5 µg total RNA are electrophoresed on a denaturing 1% formaldehyde-agarose gel, transferred to a NYTRAN NY 13 N membrane (Schleicher & Schuell, Dassel, Germany) according to standard procedures (Anonymous1994), and cross-linked using a Stratalinker (Stratagene, La Jolla, USA). Equal loading and blotting of the RNA is confirmed and documented by staining with 0,03% methylenblue in 0,3M sodium acetate, pH 5,2. Membranes are pre-hybridized for 1 h at 68°C in QuickHyb Solution (Stratagene). Membranes are hybridized for 2 hours at 68°C with an *Eco*RI restriction fragment composed of nucleotides 1410 to 2340 of the bcl-2 complementary DNA (cDNA), or an *Eco*RI / *Sma*I restriction fragment composed or nucleotides 77 to 373 of the bcl-xL cDNA, which have been radiolabelled with ^{32}P by use of random hexanucleotide primers (Prime-a-Gene Labelling System, Promega Corp, Madison, USA). Hybridization with a 1.3 kb *Eco*RI / *Hind*III restriction fragment of the 3.93 kb full-length chicken-actin cloned into plasmid pUC9 as described (Miyashita et al., 1994), or a 900 bp *Apa*I restriction fragment of the 1.2 kb human GAPDH cloned into plasmid pBlueskript KS (+) is used as probe for reference. Blots are exposed to a storage phosphor screen scanned by use of a phosphorimager (Molecular Dynamics, Sunnyvale, CA). Relative messenger RNA levels are quantified by use of the ImageQuant software (Molecular Dynamics).

As a result, OD1 efficiently reduces the level of the bcl-2 mRNA and, despite the 3-base mismatches compared with the natural base sequence of bcl-xL mRNA, also effectively reduces the level of the bcl-xL mRNA. This finding clearly indicates that OD1 can tolerate mismatches, in particular those which comprise an A to G, a T to C, and a G to T substitution at positions 3, 5, and 15 of OD1, respectively, said oligonucleotide derivative still being effective in modulating the biosynthesis both of bcl-xL and bcl-2. On the other hand, OD2 only downregulates bcl-xL expression and does not reduce the level of the bcl-2 mRNA species. As expected for the control oligonucleotide derivative CO2, it selectively downregulates the expression of bcl-2.

Example 5: Western blot analysis using OD1 and OD2

To confirm the findings of the Northern blot analyses Western blot analyses of bcl-2 and bcl-xL protein levels are performed after treatment of SW2 cells with OD1 or OD2 at a concentration of 150 nM.

For each treatment, 14 ml of SW2 cells/oligonucleotide mixture are plated in a 25 cm^2 flask and incubated for 48 h under cell culture conditions. Soluble protein extracts are prepared as described (M.

Hanada et al., Cancer Res. 53 (1993), 4978 - 4986; A. Ziegler et al., J. Natl. Cancer Inst. 89 (1997), 1027 - 1036), and protein concentrations are determined using the BCA protein assay reagent (Pierce, Rockford, IL). One hundred µg of soluble protein per sample are separated on a 12% polyacrylamide SDS gel at 50 V for about 16 h. Electrotransfer to a polyvinylidene fluoride membrane (Immobilon P, Millipore, Bedford, USA) is performed in a semi-dry blotting chamber (Schleicher and Schuell) at 1 mA/cm^2 for 1 h. The blots are blocked in Tris-buffered saline containing 5% bovine serum albumin and 5% non-fat milk, and then incubated overnight at 4 °C with 200 ng/mL mouse anti-human bcl-2 monoclonal antibody (Dako Diagnostics AG, Glostrup, Denmark) or 250 ng/ml anti bcl-xL rabbit polyclonal serum (Transduction Laboratories, Lexington, USA). To detect the primary antibody, blots are incubated with a rabbit anti-mouse immunoglobulin peroxidase conjugate, or a goat anti-rabbit immunoglobulin peroxidase conjugate (Sigma Chemical Co., St. Louis, MO) for 2 h at room temperature. Visualization of the immunocomplex is achieved by enhanced chemiluminescence using the ECL™ kit (Amersham), followed by exposure to x-ray film (Fuji RX). Relative protein levels are quantified after scanning of the films using a flat bed scanner (Hewlett Packard ScanJet IIcx) and the ImageQuant software (Molecular Dynamics).

In agreement with the findings obtained on the mRNA level, the Western blot results indicate that OD1 also efficiently downregulates the expression of both the bcl-2 and the bcl-xL protein. In contrast, OD2 efficiently downregulates only the bcl-xL protein.

Example 6: **Western Blot analysis using the 2'-methoxyethoxy oligonucleotide derivatives OD3, OD4 and OD5**

The effect of the 2'-methoxyethoxy modified oligonucleotide derivatives OD3, OD4 and OD5 on bcl-2 and bcl-xL gene expression is tested as described for the unmodified oligonucleotide derivatives OD1 and OD2 (above), using SW2 SCLC cells as target cells expressing equal levels of the bcl-2 and bcl-xL proteins. SW2 cells are suspended in RPMI1640 cell culture medium and the oligonucleotide derivatives OD3, OD4 and OD 5, respectively, are added at a final concentration of 600 nM in the presence of the cationic lipid DOTAP (N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methylsulfate). The cells are allowed to incubate with the oligonucleotide derivatives for 24 - 48 hours and are then subjected to Western Blot analysis as described above.

As determined by Western blot analysis 48 h after transfection the antisense oligonucleotide derivative OD3 reduces the level of the bcl-2 protein to 40.1% and of the bcl-xL protein to 32.6% of the respective untreated controls. OD5 reduces the level of the bcl-2 protein to 48.3% and of the bcl-xL protein to 29.1%. For comparison OD4 reduces only the level of bcl-xL to 53%. The scrambled control sequence CO3 does not reveal a repressive effect on the expression of the two anti-apoptotic proteins bcl-2 and bcl-xL.

These data demonstrate the bispecificity of the oligonucleotide derivatives OD3 and OD5 on bcl-2 and bcl-xL expression, and quantitatively correlate with the numbers of base-mismatches on the two distinct mRNAs. Apparently, the 3- and 2- base-mismatches of OD3 and OD5, respectively, on the bcl-xL mRNA can be well compensated by the 2'-methoxyethoxy modifications. In contrast, the 3 base mismatches of OD4 on the bcl-2 mRNA cannot be compensated by this modification.

In a similar experiment on H125 NSCLC, specifically OD4 reduces the level of bcl-xL expression by more than 90%, but does not alter bcl-2 expression. The control oligonucleotide derivative CO4 does not show an effect on bcl-xL protein expression.

Example 7: **Caspase-3 activation assay following treatment with the 2'-methoxyethoxy modified oligonucleotide derivatives OD3, OD4 and OD5**

Caspase activity in cells is analyzed by use of a colorimetric test system. SW2 cells are treated with the 2'-methoxyethoxy modified oligonucleotide derivatives OD3, OD4 and OD5, respectivley, as described above. The cells are lysed in buffer by freeze/thawing essentially as described (Olie et al., Curr. Biol. 8 (1998), pp.955 - 958) and lysates are centifuged at 13000 rpm at 4°C for 15 min. Cytosolic protein is mixed with 50 µM of the caspase-3 specific substrate DEVC-pNA (Asp-Glu-Val-Asp-p-nitroanilides) (Bachem, Dübendorf, Switzerland) and incubated at 37°C. Subsequently, substrate cleavage is monitored at 405nm using a SPECTRAmax 340 microplate reader and analyzed using SOFTmax PRO software (Molecular Devices).

As determined in such a caspase activation assay, 24h after transfection with the antisense oligonucleotide derivatives OD3, OD4 or OD5, respectively, there is no evidence of caspase activation. Following 48 h transfection, however, there is more than a 2-fold increase in caspase activation when cells have been treated with the bispecific oligonucleotide derivatives OD3 or OD5, respectively. No effect on caspase activation is observed with the bcl-xL specific antisense oligonucleotide derivative OD4 nor with the scrambled sequence control CO3. This result suggests that in SW2 cells repression of bcl-2 expression, modulated by OD3 and OD5, respectively, rather than repression of bcl-xL expression induces apoptosis and that these SCLC cells are dependent on the bcl-2 protein for survival.

In a similar experiment, performed with OD4 on H125 NSCLC, OD4 induces apoptosis. The control oligonucleotide CO4 does not show an effect on the tumor cells. This indicates that OD4 efficiently and specifically tackles bcl-xL expression.

Example 8: Determination of the viability of lung cancer cell lines after treatment with OD1 or OD2

The SW2 SCLC and the H125 NSCLC cell lines both co-express bcl-2 and bcl-xL, although bcl-2 expression in H125 cells is rather low. This, however, does not necessarily mean that both death antagonists are equally important survival factors for the different lung cancer cell types. To compare the effects of downregulating bcl-2 and/or bcl-xL, SW2 and H125 cells are treated with the antisense oligonucleotide derivatives OD1 or OD2, and for control purposes, with CO1 or CO2, and the cytotoxic effects are measured in colorimetric cell viability assays.

For SW2 cells a test system which is based on the cleavage of the tetrazolium salt WST-1 (Boehringer Mannheim) by mitochondrial dehydrogenases in viable cells (Cory et al., 1991) is used. For each treatment with oligonucleotide derivatives, 100 µL of cells/oligonucleotide derivative mixture are plated in triplicates in 96-well plates. Cells are incubated for 96 h under cell culture conditions, and then 10 µL of WST-1 reagent are added per well and allowed to react for 3 h at 37°C. Absorbance at 450 nm is measured by use of an enzyme-linked immunosorbent assay reader (2550 EIA reader, Bio Rad Laboratories Ltd., Hercules, CA).

The viability of H125 cells is determined by use of a standard MTT. For each treatment, 100 µL of cells/oligonucleotide derivative mixture are plated in triplicates in 96-well plates. Cells are incubated for 96 h under cell culture conditions before 10 µL of MTT reagent are added per well and allowed to react for 2 h. Cells are lysed for 3 hours under 37°C with 100 µl lysis buffer, containing 20% SDS in 50% dimethyl-formamide and 50% H_2O, pH 4,7. Absorbance at 570 nm is measured by use of an enzyme-linked immunosorbent assay reader (2550 EIA reader, Bio Rad Laboratories Ltd., Hercules, CA). Data are expressed as percent of the absorbance of untreated control cells.

As a result, the viability of SW2 cells is strongly reduced by treatment with the bi-specific oligonucleotide derivative OD1 and the bcl-2-specific control oligonucleotide derivative CO2. In contrast, treatment with the bcl-xL-specific oligonucleotide derivative OD2 is not effective when compared with the SC1 control oligonucleotide derivative. This indicates that bcl-2 but not bcl-xL is the critical survival factor for this lung cancer cell line.

In contrast to the SW2 cell line, the H125 cell line is highly susceptible to treatment with oligonucleotide derivative OD2 and also to treatment with oligonucleotide derivative OD1. Since the control oligonucleotide derivative CO2 is less effective in reducing the viability of these cells, it can be concluded that bcl-xL is the more critical survival factor for this NSCLC cell line and that bcl-2 only plays a minor protective role.

Example 9: Detection of apoptotic cells based on light scattering properties and electron microscopy

To demonstrate that death of SW2 cells after treatment with oligonucleotide derivative OD1 is apoptotic by nature, flow cytometric analysis of cell morphology based on their light scattering properties according to the method of Cotter et al., 1992.

Approximately 0.2 x 10^6 of ODN-treated cells are fixed in 2% paraformaldehyde, permeabilized in 0.05% Triton-X100 and measured by use of a FACSCalibur cytofluorometer (Becton Dickinson, Mountain View, CA).

Cells showing changes in light scattering are also subjected to electron microscopy. Briefly, for electron microscopic examination SW2 cells are fixed in 2,5% glutaraldehyde, 0,8% paraformaldehyde in 0,05M Na-Cacodylate buffer. For transmission electron microscopy, cells are dehydrated in ethanol and propylene oxide. After dehydrating, cells are embeded in Epon 812, cut into 60nm slices, contrasted with Reynolds Solution (uranyl acetate and lead nitrate), and visualized by use of a Philips CM 420 electron microscope. For scanning electron microscopy, cells are dried in aceton, stained with gold and visualized by use of a Philips SEM 505.

Treatment of cells with 150 nM OD1 for 3 days results in extensive plasma membrane blebbing which is considered as a hallmark of apoptosis. The FSC/SSC analysis shows that this membrane blebbing also dramatically increases the side-light scattering intensities of the cells.

Claims

1. An oligonucleotide derivative which is specifically hybridizable to a region ranging from base position no. 687 (5') to no. 706 (3') of the human bcl-xL mRNA encoding human bcl-xL protein.

2. The oligonucleotide derivative according to claim 1, which oligonucleotide derivative additionally is specifically hybridizable to a region ranging from base position 2032 (5') to 2051 (3') of the human bcl-2 mRNA encoding human bcl-2 protein.

3. The oligonucleotide derivative according to any of claims 1 or 2, comprising a base sequence which is complementary to at least a part of the said region of the human bcl-xL mRNA or the human bcl-2 mRNA, or wherein such base sequence contains up to 3 mispairing building blocks, or wherein such base sequence contains up to 3 abasic building blocks.

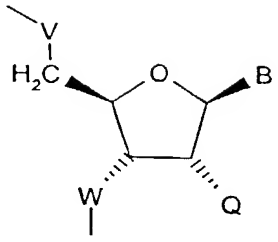
4. The oligonucleotide derivative according to any of claims 1 to 3 having a length of 8 to 25 consecutive building blocks.

5. The oligonucleotide derivative according to claim 4 having a length of 20 consecutive building blocks.

6. The oligonucleotide derivative according to any of claims 3 to 5, wherein said base sequence is selected from the group consisting of the base sequence 5'-AAGGCATCCCAGCCTCCGTT-3' (SEQ ID NO: 3), the base sequence 5'-AAAGTATCCCAGCCGCCGTT-3' (SEQ ID NO: 4), and the base sequence 5'-AAAGCATCCCAGCCTCCGTT-3' (SEQ ID NO: 5).

7. The oligonucleotide derivative according to any of claims 1 to 6, consisting of a base sequence selected from the group consisting of the base sequence 5'-AAGGCATCCCAGCCTCCGTT-3' (SEQ ID NO: 3), the base sequence 5'-AAAGTATCCCAGCCGCCGTT-3' (SEQ ID NO: 4) and the base sequence 5'-AAAGCATCCCAGCCTCCGTT-3' (SEQ ID NO: 5).

8. The oligonucleotide derivative according to any of claims 1 to 7, comprising at least one building block of formula (I)



(I),

wherein

Q is H, $-OCH_3$, $-O(CH_2CH_2)_nOCH_3$, or $-OCH_2CH_2NR_1R_2$, wherein R_1 and R_2 are, independently of each other, H or $-CH_3$, and wherein n is 1, 2 or 3;

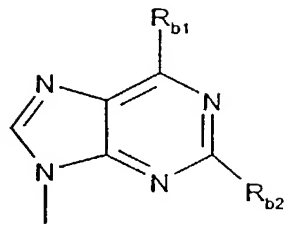
V and W are, independently of each other, the same or different radicals of an internucleosidic bridging group selected from the following group: 5'-O-P(O)(OH)-O-3' (phosphodiester), 5'-O-P(O)(SH)-O-3' (phosphorothioate), 5'-O-P(S)(SH)-O-3' (phosphodithioate), 5'-O-P(O)(CH_3)-O-3' (methylphosphonate), 5'-O-P(O)(NH-R_7)-O-3' (phosphoamidate) in which R_7 is C_1-C_3alkyl, 5'-O-P(O)(OR_8)-O-3' (phosphotriester) in which R_8 is C_1-C_3alkyl, 5'-O-$S(O)_2$-CH_2-3' (sulfonate), 5'-O-$S(O)_2$-NH-3' (sulfamate), 5'-NH-$S(O)_2$-CH_2-3' (sulfonamide), 5'-CH_2-$S(O)_2$-CH_2-3' (sulfone), 5'-O-S(O)-O-3' (sulfite), 5'-CH_2-S(O)-CH_2-3' (sulfoxide), 5'-CH_2-S-CH_2-3' (sulfide), 5'-O-CH_2-O-3' (formacetal), 5'-S-CH_2-O-3' (3'-thioformacetal), 5'-O-CH_2-S-3' (5'-thioformacetal), 5'-CH_2-CH_2-S-3' (thioether), 5'-CH_2-NH-O-3' (hydroxylamine), 5'-CH_2-N(CH_3)-O-3' (methylene(methylimino)), 5'-CH_2-O-N(CH_3)-3' (methyleneoxy(methylimino)), 5'-O-C(O)-NH-3' (5'-N-carbamate), 5'-CH_2-C(O)-NH-3' (amide), 5'-NH-C(O)-CH_2-3' (amide 2), 5'-CH_2-NH-C(O)-3' (amide 3) and 5'-C(O)-NH-CH_2-3' (amide 4), and the tautomeric forms thereof;

or one of V and W is such an internucleosidic bridging group and the other is a terminal radical selected from the group consisting of -OH and $-NH_2$, preferably -OH; and

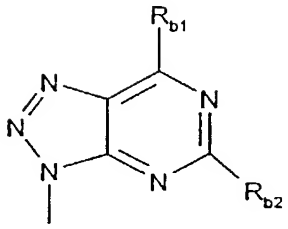
B is a radical of a nucleic acid base;

with the proviso that if Q is H, then at least one of V or W is an internucleosidic bridging group other than 5'-O-P(O)(OH)-O-3' (phosphodiester).

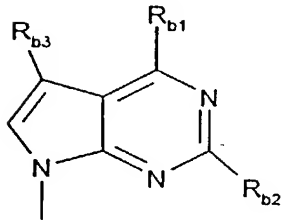
9. An oligonucleotide derivative according to claim 8, wherein
B is a radical of the formula (V1), (V2), (V3), (V4) or (V5)



(V1),



(V2),



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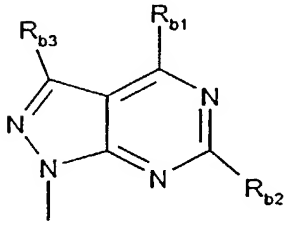
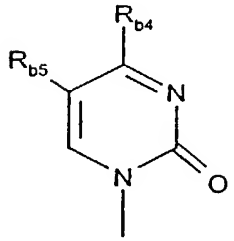


(V4),



(V5)

in which

R_{b1} is $-NH_2$, -SH or -OH;

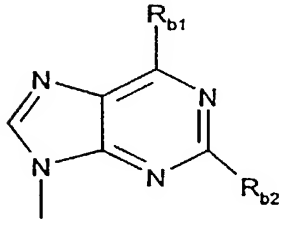
R_{b2} is H, $-NH_2$ or -OH; and

R_{b3} is H, Br, I, -CN, $-C\equiv C-CH_3$, $-C(O)NH_2$ or $-CH_3$;

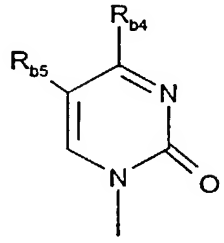
R_{b4} is $-NH_2$ or -OH; and

R_{b5} is H, F, Br, I, -CN, $-C\equiv C-CH_3$, $-C(O)NH_2$ or $-CH_3$.

10. An oligonucleotide derivative according to claim 9, wherein B is a radical of the formula (V1) or (V5)



(V1),



(V5)

in which

R_{b1} is $-NH_2$, -SH or -OH;

R_{b2} is H, $-NH_2$ or -OH;

R_{b4} is $-NH_2$ or -OH; and

R_{b5} is H, F, Br, I, -CN, $-C\equiv C-CH_3$, $-C(O)NH_2$ or $-CH_3$.

11. An oligonucleotide derivative according to claim 10, wherein B is selected from the group of the following radicals: xanthine, hypoxanthine, adenine, 2-aminoadenine, guanine, 6-thioguanine, uracil, thymine, cytosine, 5-methylcytosine, 5-propynyluracil, 5-fluorouracil and 5-propynylcytosine.

12. An oligonucleotide derivative according to claim 8, wherein V and W, as radicals of an internucleosidic bridging group, are, independently of each other, selected from the following group: 5'-O-P(O)(OH)-O-3' (phosphodiester), 5'-O-P(O)(SH)-O-3' (phosphorothioate) and 5'-CH_2-C(O)-NH-3' (amide).

13. An oligonucleotide derivative according to claim 12, wherein one of the radicals V or W, as radicals of an internucleosidic bridging group, is 5'-O-P(O)(OH)-O-3' (phosphodiester) and the other radical is 5'-O-P(O)(SH)-O-3' (phosphorothioate).

14. The oligonucleotide derivate according to claim 13, wherein both V and W as radicals of an internucleosidic bridging group are 5'-O-P(O)(OH)-O-3' (phosphodiester) or are 5'-O-P(O)(SH)-O-3' (phosphorothioate).

15. The oligonucleotide derivative according to claim 8, wherein V and W, as terminal radicals, are, independently of each other, -OH or $-NH_2$.

16. The oligonucleotide derivative according to claim 8, wherein Q is selected from the group consisting of 2'-O-methyl, 2'-O-methoxyethoxy, 2'-O-di(methoxyethoxy), 2'-O-tri(methoxyethoxy), 2'-O-aminoethoxy, 2'-O-monomethylaminoethoxy and 2'-O-dimethylaminoethoxy.

17. The oligonucleotide derivative according to claim 8, consisting of a base sequence selected from the group consisting of the base sequence 5'-AAGGCATCCCAGCCTCCGTT-3' (SEQ ID NO: 3) and the base sequence 5'-AAAGTATCCCAGCCGCCGTT-3' (SEQ ID NO: 4), wherein each V and each W as radicals of an internucleosidic bridging group of the building blocks according to formula (I) are of the 5'-O-P(O)(SH)-O-3' (phosphorothioate) type and wherein each Q according to formula (I) is -H.

18. The oligonucleotide derivative according to claim 8, consisting of a base sequence selected from the group consisting of the base sequence 5'-AAGGCATCCCAGCCTCCGTT-3' (SEQ ID NO: 3), the base sequence 5'-AAAGTATCCCAGCCGCCGTT-3' (SEQ ID NO: 4), and the base sequence 5'-AAAGCATCCCAGCCTCCGTT-3' (SEQ ID NO: 5), wherein each V and each W as radicals of an

internucleosidic bridging group of all building blocks according to formula (I) are of the 5'-O-P(O)(SH)-O-3' (phosphorothioate) type, and wherein each Q according to formula (I) of the nucleotides being underlined is $-OCH_2CH_2OCH_3$ and wherein each Q according to formula (I) of the remaining nucleotides is -H.

19. A process for the preparation of an oligonucleotide derivative according to any of claims 1 to 18, said process comprising incorporating at least one building block of formula (I) according to claim 8 into the oligonucleotide derivative during oligonucleotide synthesis.

20. A pharmaceutical composition comprising an oligonucleotide derivative according to any of claims 1 to 18, optionally together with a pharmaceutically acceptable excipient and/or auxilliary substance, said pharmaceutical composition being suitable for administration to humans suffering from a disease that responds to the modulation of human bcl-xL expression or that responds to the modulation of human bcl-xl and human bcl-2 expression.

21. An oligonucleotide derivative according to any of claims 1 to 18 for use in medicine.

22. Use of an oligonucleotide derivative according to any of claims 1 to 18 in the preparation of a pharmaceutical composition for treatment of a disease status associated with the biosynthesis of human bcl-xL protein or with the biosynthesis of both the human bcl-xL protein and the human bcl-2 protein.

23. A method of treatment of a disease status accociated with the expression of human bcl-xL protein or with the expression of both the human bcl-xL protein and the human bcl-2 protein, comprising application of an oligonucleotide derivative according to any of claims 1 to 18.

24. A method of modulating the biosynthesis of human bcl-xL protein in a cell, comprising application of an oligonucleotide derivative according to any of claims 1 to 18 to said cell.

25. An oligonucleotide derivative according to any of claims 1 to 18 for use in a diagnostic method.

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(54) Title: OLIGONUCLEOTIDE DERIVATIVES DIRECTED AGAINST HUMAN BCL-XL AND HUMAN BCL-2 MRNA

(57) Abstract: The present invention relates to antisense oligonucleotide derivatives directed against human bcl-xL mRNA and being capable of modulating the biosynthesis of human bcl-xL protein. Furthermore, the present invention relates to antisense oligonucleotide derivatives directed against both human bcl-xL nRNA and human bcl-2 mRNA, and being capable of modulating the biosynthesis of both human bcl-xl protein and human bcl-2 protein. The present invention further relates to a pharmaceutical composition comprising such oligonucleotide derivatives, uses thereof and methods of treatment and diagnosis utilizing such oligonucleotide derivatives.

DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled "**OLIGONUCLEOTIDE DERIVATIVES DIRECTED AGAINST HUMAN BCL-2 AND HUMAN BCL-X MRNA,**" the specification of which (check one): ☐ is attached hereto; ☒ was filed on October 30, 2001 as Application Serial No. 10/018,437 as a U.S. National Phase of PCT International Application No. PCT/EP00/03708, filed April 26, 2000. I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above. I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

(Application Serial Number)	(Country)	(Day/Month/Year Filed)	Priority Claimed ☒ Yes	☐ No
GB 99 10119.8	Great Britian	April 30, 1999		
			☐ Yes	☐ No

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled "**OLIGONUCLEOTIDE DERIVATIVES DIRECTED AGAINST HUMAN BCL-2 AND HUMAN BCL-X MRNA**," the specification of which (check one): ☐ is attached hereto; ☒ was filed on October 30, 2001 as Application Serial No. 10/018,437 as a U.S. National Phase of PCT International Application No. PCT/EP00/03708, filed April 26, 2000. I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above. I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56.

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POWER OF ATTORNEY: I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecu this application and transact all business in the Patent and Trademark Office connected therewith:

28 –

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Date ☒	Signature ☒

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State or Country Germany	State or Country Germany
Date ☒ March, 26, 2002	Signature ☒ D. Hüsken

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